TITLE OF THE INVENTION

BIOCONJUGATES AND DELIVERY OF BIOACTIVE AGENTS

BACKGROUND OF THE INVENTION

The present invention relates to bioconjugates and the delivery of bioactive agents which are preferably targeted for site-specific release in cells, tissues or organs. More particularly, this invention relates to bioconjugates which comprise a bioactive agent and an organocobalt complex. The bioactive agent is covalently bonded directly or indirectly to the cobalt atom of the organocobalt complex. The bioactive agent is released from the bioconjugate by the cleavage of the covalent bond between the bioactive agent and the cobalt atom in the organocobalt complex. The cleavage may occur as a result of normal displacement by cellular nucleophiles or enzymatic action, but is preferably caused to occur selectively at a predetermined release site by application of an external signal. The external signal may be light or photoexcitation, i.e. photolysis, or it may be ultrasound, i.e. sonolysis. Further, if the photolysis takes place in the presence of a magnetic field surrounding the release site, the release of the bioactive agent into surrounding healthy tissue is minimized.

The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated by reference, and for convenience are referenced in the following text by author and date and are listed alphabetically by author in the appended bibliography.

The focus of a substantial body of research has been the development of a system whereby a pharmaceutical agent can be selectively delivered to a desired anatomic location; namely the site in need of treatment. In spite of the great progress which has been achieved in this regard, many pharmaceutical delivery systems for the treatment of various diseases or health risks, e.g., the treatment of cancer, impart substantial risk to the patient. With respect to the treatment of cancer, drugs which are effective in attacking malignant cells to destroy them, or at least limit their proliferation, have a tendency to attack benign cells also. Therefore, it is highly desirable to limit the location of their action to that of the malignancy, and to ensure that at any particular time effective, but not excessive, amounts of such drugs are used.

Although it is desired to concentrate a cytotoxic agent at a targeted site, current cancer treatment protocols for administering these cytotoxic agents typically call for repeated intravenous dosing, with careful monitoring of the patient. The drugs are often used in combination to exert a multi-faceted assault on neoplastic cells. The dose is selected to be just below the amount that will produce acute (and sometimes chronic) toxicity that can lead to life-threatening cardiomyopathy, myelotoxicity, hepatic toxicity, or renal toxicity. Alopecia (hair loss), mucositis, stomatitis, and nausea are other common, but generally not life-threatening, side effects at these doses. Since many of these compounds are potent vesicants, tissue necrosis will occur if localized extravasation (loss of the drug from blood to the surrounding tissue) occurs. These effects occur since the blood generally attains a specified concentration of that drug before becoming effective. Because the blood is transported throughout the body of the host being treated, so is the pharmaceutical agent. Following this technique provides an even distribution of the drug throughout the body, rather than concentrating it at the treatment site. Moreover, such systemic treatment methods expose the healthy cells to the cytotoxic agent concurrent with the treatment of the unhealthy or diseased cells besides limiting the concentration of the drug at the site where it is most needed.

Previous attempts to administer such drugs by direct injection into the location of the organ having the malignancy are only partially effective, because of migration of the drug from that location and as a result of extensive tissue necrosis from extravasation. Such dispersion cannot be totally prevented, with the result that excessive quantities of drug need to be administered to attain a desired result. Although careful clinical monitoring may prevent extensive damage or loss of viable tissue, the providing of a pharmaceutical agent-carrier system which is actively transported through standard biological systems to the treatment site prior to activation of the pharmaceutical agent would be highly desirable not only in optimizing utilization of the drug but also in the reduction of side effects and/or the minimization of the destruction of healthy cells. The direct injection of cytotoxic agents into solid tumors of the breast, bladder, prostate and lung using conventional cytotoxic chemotherapeutic agents as adjuvants to surgery and/or radiotherapy has been of limited success in prolonging the lives of patients. This is partially due to the dose limitations imposed by the acute and chronic toxicity to tissues or organ systems beyond those that are targeted.

As it relates to the administration of cytotoxic or antineoplastic drugs, the effective resolution of concerns relating to modes of administration, to the limitation of dosage size and frequency of administration, and to side effects would certainly be of benefit to the treatment of cancer.

Oligonucleotides that specifically interfere with gene expression at the transcriptional or translational levels have the potential to be used as therapeutic agents to control the synthesis of deleterious proteins associated with viral, neoplastic or other diseases. It is possible to select single-stranded oligonucleotides that recognize and bind to the major groove of a stretch of double-stranded DNA in a sequence-specific manner to form a triple helix (Le Doan et al., 1987; Moser and Dervan, 1987). Triple helix-forming oligonucleotides targeted to the promoter region of certain genes have been used to physically block RNA synthesis in cell-free transcription assays (Cooney et al., 1988; Postel et al., 1992; Skoog et al., 1993; Rando et al., 1994). Similarly, *in vitro* translation assays have been used to demonstrate that antisense oligonucleotides can bind mRNA targets and prevent protein synthesis (Uhlmann and Peyman, 1990; Cohen and Hogan, 1994).

Although antisense oligonucleotides have shown great efficacy in the selective inhibition of gene expression (Stein and Cohen, 1988; Szczylik et al., 1991; Gray et al., 1993), the therapeutic applications of such antisense oligonucleotides are currently limited by their low physiological stability, slow cellular uptake, and lack of tissue specificity. The instability obstacles have been largely overcome by use of backbone-modified oligonucleotides that are more resistant to nucleases. Methylphosphonates, protein-nucleic acid conjugates, and phosphorothioates all appear to resist enzymatic digestion better than the corresponding natural oligonucleotides (Chang and Miller, 1991; Wickstrom et al., 1992; Letsinger, 1993; Zon, 1993).

Problems with cellular uptake of antisense oligonucleotides have been more difficult to solve. Endogenous uptake pathways that rely on pinocytosis and related processes generally have insufficient capacity to deliver the quantities of antisense of oligonucleotides required to suppress gene expression (Vlassov et al., 1994). Hydrophobic modifications have also been undertaken to improve membrane permeability, but such derivatization strategies are most useful only for short oligonucleotides (Vlassov et al., 1994). Although complexes of antisense constructs with cationic liposomes or immunoliposomes (Gao and Huang, 1991; Bennett et al., 1992, Ma and Wei, 1996) and polylsine (Trubetskoy et al., 1992; Bunnell et al., 1992) have significantly